CLAIMS

What is claimed is:

1. A formulation comprising a gas microsphere liposome composite suspended in a medium, wherein the gas microsphere liposome composite comprises:

a gas-filled microsphere;

at least one of a lipid and a surfactant adsorbed onto the surface of the gas-filled microsphere; and

liquid-filled liposomes attached to the lipid or surfactant.

2. The formulation of claim 1 wherein the gas of the gas-filled microsphere has a solubility of less than about 1.0% (v/v) in water at 25°C and 1 atm.

3. The formulation of claim 1 wherein the gas-filled microsphere has an average diameter of about 0.1μm to about 10μm.

4. The formulation of claim 1 wherein the gas-filled microsphere has an average diameter of about 0.5μm to about 10μm.

5. The formulation of claim 1 wherein the gas-filled microsphere comprises at least one inert gas.

6. The formulation of claim 5 wherein the inert gas is a noble gas.

7. The formulation of claim 5 wherein the inert gas is a perfluoroether gas.

8. The formulation of claim 5 wherein the inert gas is a perfluorocarbon gas.

9. The formulation of claim 1 wherein the gas-filled microsphere has a lipid adsorbed onto the surface of the gas-filled microsphere.

10. The formulation of claim 1 wherein the gas-filled microsphere has a surfactant adsorbed onto the surface of the gas-filled microsphere.

11. The formulation of claim 1 wherein the lipid or surfactant forms a mono-molecular layer on the surface of the gas-filled microsphere.

12. The formulation of claim 1 wherein the lipid or surfactant forms a bi-molecular liposomal layer or multi-molecular liposomal layer on the surface of the gas-filled microsphere.

13. The formulation of claim 1 wherein the surfactant is a non-ionic surfactant, cationic surfactant, or anionic surfactant.

14. The formulation of claim 13 wherein the non-ionic surfactant comprises polyethylene glycol, polypropylene glycol, polyvinylpyrollidone, polyvinylalcohol, cellulose, gelatin, xanthan gum, pectin, or dextran.

15. The formulation of claim 13 wherein the cationic surfactant comprises a tetraalkyl ammonium, tetraalkyl phosphonium ion, or a suitable salt thereof.

16. The formulation of claim 15 wherein the cationic surfactant comprises a tetrahexyl ammonium, tetraoctyl ammonium, tetradecyl ammonium, tetrabutyl ammonium, tetrahexyl phosphonium, tetraoctyl phosphonium, tetrabutyl phosphonium, tetraphenyl phosphonium, or a suitable salt thereof.

17. The formulation of claim 13 wherein the anionic surfactant comprises an alkyl sulfonate, an alkyl carboxylate, or a suitable salt thereof.

18. The formulation of claim 17 wherein the anionic surfactant comprises dodecyl sulfate, palmityl sulfate, dodecyl carboxylate, palmityl carboxylate, or a suitable salt thereof.

19. The formulation of claim 1 wherein the lipid comprises a phospholipid, glycolipid, triglyceride or fatty acid.

20. The formulation of claim 19 wherein the phospholipid comprises dipalmitoylphosphatidyl choline, dimyristoylphosphatidyl choline, dilauryoylphosphatidyl choline, or dioleoylphosphatidyl choline.

21. The formulation of claim 1 wherein the liquid-filled liposomes are attached to the adsorbed lipid or surfactant in a continuous fashion.

22. The formulation of claim 1 wherein the liquid-filled liposomes occupy greater than about 50% of the outer surface of the gas-filled microsphere area.

23. The formulation of claim 1 wherein each of the liquid-filled liposomes independently has a diameter of about 10nm to about 200nm.

24. The formulation of claim 1 wherein each of the liquid-filled liposomes independently has a diameter of about 20nm to about 100nm.

25. The formulation of claim 1 wherein each of the liquid-filled liposomes independently has a diameter that is less than about 10% of the diameter of the gas-filled microsphere.

26. The formulation of claim 1 wherein each of the liquid-filled liposomes independently comprises a therapeutic agent or diagnostic agent in the interior of the liquid-filled liposomes.

27. The formulation of claim 26 wherein the therapeutic agent is an anticoagulant, thrombolytic, antineoplastic agent, or anti-inflammatory agent.

28. The formulation of claim 26 wherein the therapeutic agent comprises doxorubicin, cyclophosphamide, adriamycin, methotrexate, gemcitabine, navelbine, cisplatin, tissue plasminogen activator, integrelin, roxifiban, methotrexate or enbrel.

29. The formulation of claim 26 wherein the diagnostic agent comprises an X-ray contrast agent or an MRI contrast agent.

30. The formulation of claim 1 wherein each of the liquid-filled liposomes independently has high affinity, targeting moieties attached to the surface of the liquid-filled liposomes.

31. The formulation of claim 30 wherein the high affinity targeting moiety attached to the surface of the gas microsphere liposome composite comprises:

a ligand which binds to a receptor which is up-regulated in angiogenesis;

a ligand which binds to a receptor which is up-regulated in inflammation; or

a ligand which binds to a receptor which is up-regulated in atherosclerosis.

32. The formulation of claim 30 wherein the high affinity targeting moiety attached to the surface of the gas microsphere liposome composite comprises:

a ligand which binds to the integrons $\alpha_v\beta_3$, $\alpha_v\beta_5$ or GpIIb/IIIa;

a ligand which binds to a matrix metalloproteinase; or

a ligand which binds to the LTB_4 receptor.

33. The formulation of claim 30 wherein the high affinity targeting moiety attached to the surface of the gas microsphere liposome composite comprises:

1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-cyclo(Arg-Gly-Asp-D-Phe-Lys)-dodecanoate;

DPPE-PEG_{3400}-cyclo(Arg-Gly-Asp-D-Phe-Lys)-dodecanoate;

1-(1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamino)-α,ω-dicarbonyl PEG_{3400}-2-{[7-(N-hydroxycarbamoyl)(3S,6R,7S)-4-aza-6-(2-methylpropyl)-11-oxa-5-oxobicyclo[10.2.2]hexadeca-1(15),12(16),13-trien-3-yl]carbonylamino}-N-(3-aminopropyl)acetamide; or

1-(1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamino)-α,ω-dicarbonyl PEG_{3400}-[7-(N-hydroxycarbamoyl)(3S,6R,7S)-4-aza-6-(2-methylpropyl)-11-oxa-5-oxobicyclo[10.2.2]hexadeca-1(15),12(16),13-trien-3-yl]-N-{[4-(aminomethyl)phenyl]methyl}carboxamide.

34. The formulation of claim 1 wherein each of the liquid-filled liposomes independently comprises liquid from the medium of suspension.

35. The formulation of claim 1 wherein the gas microsphere liposome composite has a mean diameter of about 0.1μm to about 10μm.

36. The formulation of claim 1 wherein the gas microsphere liposome composite has a mean diameter of about 0.2μm to about 4μm.

37. The formulation of claim 1 wherein the gas microsphere liposome composite exists as an aggregate of two or more gas microsphere liposome composites.

38. The formulation of claim 37 wherein the aggregate has a diameter of about 1μm to about 100μm.

39. The formulation of claim 1 wherein the gas microsphere liposome composite has a density of about 0.90 to about 1.10 of the density of the medium.

40. The formulation of claim 1 wherein the lipid or surfactant comprises a high affinity targeting moiety.

41. The formulation of claim 1 wherein the lipid or surfactant comprises a therapeutic agent.

42. The formulation of claim 41 wherein the therapeutic agent is doxorubicin, cyclophosphamide, adriamycin, methotrexate, gemcitabine, navelbine, cisplatin, tissue plasminogen activator, integrelin, roxifiban, methotrexate or enbrel.

43. The formulation of claim 1 wherein the medium comprises a diagnostic agent.

44. The formulation of claim 43 wherein the diagnostic agent is an X-ray or MRI contrast agent.

45. The formulation of claim 40 wherein the high affinity targeting moiety comprises:

1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-cyclo(Arg-Gly-Asp-D-Phe-Lys)-dodecanoate;

DPPE-PEG_{3400}-cyclo(Arg-Gly-Asp-D-Phe-Lys)-dodecanoate;

1-(1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamino)-α,ω-dicarbonyl PEG_{3400}-2-{[7-(N-hydroxycarbamoyl)(3S,6R,7S)-4-aza-6-(2-methylpropyl)-11-oxa-5-oxobicyclo[10.2.2]hexadeca-1(15),12(16),13-trien-3-yl]carbonylamino}-N-(3-aminopropyl)acetamide; or

1-(1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamino)-α,ω-dicarbonyl PEG_{3400}-[7-(N-hydroxycarbamoyl)(3S,6R,7S)-4-aza-6-(2-methylpropyl)-11-oxa-5-oxobicyclo[10.2.2]hexadeca-1(15),12(16),13-trien-3-yl]-N-{[4-(aminomethyl)phenyl]methyl}carboxamide.

46. A method of ultrasound imaging in a patient in need of such ultrasound imaging comprising:

administering to the patient an effective amount of a formulation of any one of claims 1-45;

allowing a sufficient period of time for the circulation of the gas microsphere composite to reach the targeted area; and

performing ultrasound imaging on the patient.

47. The method of claim 46 wherein the patient is a human.

48. The method of claim 46 wherein the effective amount of the formulation comprises about 10^3 to about 10^{10} gas microsphere liposome composites.

49. The method of claim 46 wherein the sufficient period of time is about 5 minutes to about 2 hours.

50. The method of claim 46 wherein the sufficient period of time is about 5 to about 30 minutes.

51. A method of treating heart disease, inflammation, infection, cancer or thromboembolic disease in a patient in need of such treatment comprising:

administering to the patient an effective amount of a formulation of any one of claims 1-45, wherein one or more of the liquid-filled liposomes independently comprises a therapeutic agent;

allowing a sufficient period of time for the circulation of the gas microsphere composite to the targeted area; and

applying ultrasound energy to the region of pathology in the patient sufficient to cause the therapeutic agent to be released from the microsphere liposome composite at the region of pathology.

52. The method of claim 51 wherein the patient is a human.

53. The method of claim 51 wherein each of the liquid-filled liposomes independently comprises a therapeutic agent.

54. The method of claim 51 wherein the effective amount of the formulation comprises about 10^3 to about 10^{10} gas microsphere liposome composites.

55. A method for preparing a formulation of any one of claims 1-45 comprising:

contacting a suspension of liposomes in a aqueous solution comprising at least one lipid or one surfactant; and

mixing the suspension with a gas that has a solubility of less than about 1.0% (v/v) in water at 25°C and 1 atm sufficient to provide the formulation.

56. The method of claim 55 wherein the mixing is accomplished by mechanical agitation.

57. The method of claim 55 wherein the mixing is accomplished by ultrasonification.

58. The method of claim 55 wherein the mixing is accomplished by high speed injection of the gas into the aqueous liposome suspension.

59. A method for preparing a formulation of any one of claims 1-45 comprising:

contacting a suspension of liposomes in a aqueous solution comprising at least one therapeutic agent and at least one surfactant; and

mixing the aqueous liposome suspension with a gas that has a solubility of less than about 1.0% (v/v) in water at 25°C and 1 atm sufficient to provide the formulation.

60. The method of claim 59 wherein the mixing is accomplished by mechanical agitation.

61. The method of claim 59 wherein the mixing is accomplished by ultrasonification.

62. The method of claim 59 wherein the mixing is accomplished by high speed injection of the gas into the aqueous liposome suspension.

63. A kit for the preparation of a formulation of any one of claims 1-45 comprising:

a container comprising an aqueous solution wherein the aqueous solution comprises at least one surfactant and liquid-filled liposomes; and

a means for introducing a gas that has a solubility of less than about 1.0% (v/v) in water at 25°C and 1 atm into the aqueous solution.

64. The kit of claim 63 wherein the container comprises a head space.

65. The kit of claim 63 wherein the head space comprises at least one inert gas having a solubility of less than about 1.0%(v/v) in water at 25°C and 1 atm pressure.

66. The kit of claim 63 wherein the inert gas is a perfluorcarbon gas.

67. The kit of claim 63 wherein the inert gas is a perfluoroether gas.

68. The kit of claim 63 wherein the inert gas is a noble gas.

69. A formulation comprising a gas microsphere liposome composite suspended in a medium, wherein the gas microsphere liposome composite comprises:

a gas-filled microsphere;

at least one of a lipid and a surfactant adsorbed onto the surface of the gas-filled microsphere; and

liquid-filled liposomes attached to the lipid or surfactant;

for use in medical therapy or diagnosis.

70. The use of a formulation comprising a gas microsphere liposome composite suspended in a medium, wherein the gas microsphere liposome composite comprises:

a gas-filled microsphere;

at least one of a lipid and a surfactant adsorbed onto the surface of the gas-filled microsphere; and

liquid-filled liposomes attached to the lipid or surfactant;

for the manufacture of a medicament for treating heart disease, inflammation, infection, cancer or thromboembolic disease in a patient in need of such treatment.

71. The use of a formulation comprising a gas microsphere liposome composite suspended in a medium, wherein the gas microsphere liposome composite comprises:

a gas-filled microsphere;

at least one of a lipid and a surfactant adsorbed onto the surface of the gas-filled microsphere; and

liquid-filled liposomes attached to the lipid or surfactant;

for the manufacture of a medicament for diagnostic imaging in a patient in need of such diagnostic imaging.

72. The use of a formulation comprising a gas microsphere liposome composite suspended in a medium, wherein the gas microsphere liposome composite comprises:

a gas-filled microsphere;

at least one of a lipid and a surfactant adsorbed onto the surface of the gas-filled microsphere; and

liquid-filled liposomes attached to the lipid or surfactant;

for the manufacture of a medicament for ultrasound imaging in a patient in need of such ultrasound imaging.